



# Clinical Chemistry Trainee Council

## Pearls of Laboratory Medicine

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**TITLE: Pseudohyponatremia**

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**Slide 1:**

Hello, my name is Yan Victoria Zhang. I am an Assistant Professor at the University of Rochester Medical Center and Director of the Clinical Mass Spectrometry and Toxicology Laboratory and Associate Director of the Hematology and Chemistry Laboratory at Strong Memorial Hospital. Welcome to this Pearl of Laboratory Medicine on “Pseudohyponatremia.”

**Slide 2:**

Sodium, one of the four major electrolytes, is very important to human bodies. Sodium is the major cation of extracellular fluids, representing approximately 90% of inorganic cations in plasma (of a total of 154 mmol per liter). Sodium, along with other electrolytes, also plays a significant role in acid-base balance, maintaining the normal distribution of water and osmotic pressures in the extracellular fluid compartments, heart and muscle function, and proper functions of various enzymes. Nearly all metabolic processes and many processes in the body, especially in the brain, nervous system, and muscles, require electrical signals for communication. Sodium plays a significant role in the generation of these electrical signals. Given its critical role, monitoring sodium concentrations is important for patient care and sodium is one of the most frequently measured analytes in the typical clinical laboratory. Sodium is also under tight homeostatic regulation with the reference interval being 135-145 mmol/L.

Hyponatremia is commonly defined as a sodium concentration less than 135 mmol/L, with possibly a small degree of variations in different laboratories. When sodium is less than 125 mmol per liter, patients start to show initial symptoms such as nausea, vomiting, and headache. When sodium concentration is less than 120 mmol per liter, patients are in critical condition with severe symptoms such as seizure, coma, and even respiratory arrest. Without timely treatment, a patient's life will be threatened. Accurate measurement of sodium becomes very important for patient management.

**Slide 3:**

However, there is a phenomenon called pseudohyponatremia, which indicates a reported low serum sodium concentration resulting from electrolytes exclusion effect by lipids or proteins in a patient's sample. When this happens, the measured sodium concentration is less than the actual in vivo sodium concentration, and this effect becomes a significant issue for methods that require sample dilution prior to analysis.

The patient still has a normal osmolality. Therefore, this is also known as isotonic hyponatremia, which is different from hypertonic hyponatremia (caused by high levels of glucose, mannitol, and/or sorbitol in patient serum) and hypotonic hyponatremia.

**Slide 4:**

There are several methods available to measure sodium concentration in serum or plasma: flame photometry, ion-selective electrodes, atomic absorption spectrophotometry, and other spectrophotometric methods.

Flame photometry is also known as flame emission spectrophotometry. It is one of the oldest methods that measures sodium concentration based on the distinct color of sodium in a flame as shown in the picture here. It has been considered the reference method for sodium measurement.

Ion-selective electrodes are the most commonly used method in the clinical laboratory. It is based on potentiometry electrochemistry methodology. There are two different approaches to using ISE, namely, direct and indirect ISE. Direct ISE analyzes the sample directly without modification, while indirect ISE requires sample dilution before analysis.

**Slide 5:**

This slide is to review the differences between ion concentration and ion activity.

Concentration is a measure of how much substance is dissolved in the solution. It's pretty straightforward and represents the amount of the substance in the total solution volume in terms of the molarity, molality, or other concentration unit that is appropriate for the specific situation. Commonly used units for electrolytes are mmol/L or mEq/L, but mmol/L is most commonly used today.

Activity is a little harder to pin down. It represents the ion's capacity to react or its effective concentration. It is typically thought of as the free or unbound ions in solution. It is based on the chemical potential. In a biological content, it refers to the ions that are dissolved in the water phase that are directly available for biological functions. The typical unit is mEq/Kg Water or mmol/Kg Water. The relationship between concentration and activity can be transformed by the activity coefficient which is primarily dependent on ionic strength in the solution. When there is no dilution, activity is directly proportional to the concentration in water phase.

**Slide 6:**

The relationship between concentration and activity, with regard to sodium, is shown in this slide.

The typical blood sample largely consists of water (approximately 93%), electrolytes such as sodium, and other solids such as proteins and lipids. If the components can be separated such as by centrifugation, water typically comprises 93% of the solution; the solids and non-water soluble particles typically comprises 7% of the solution. Sodium and other electrolytes, however, are only present in the water phase, as shown in diagram on the right side.

This typical sample with a sodium concentration of 143 mmol per liter, as in the overall sample volume, will have an equivalent sodium activity of 154 mmol per kilogram water. The concentration is what is measured by flame photometry, and direct ion-selective electrode measures sodium activity.

Physiologically, ionic activity is considered to be more relevant than concentration to chemical equilibrium and biological processes. However, in practice, concentration is the more familiar term and forms the basis of reference intervals and medical decisions. The measured activity is conventionally converted to concentrations for final report.

**Slide 7:**

Given that ion selective electrodes measure ion activity, the question is why the measured sodium concentration equals to 143 mmol per liter instead of 154 mmol per kilogram from ion-selective electrode methodology.

Due to the different available options for reporting, there was a need to standardize terminology and processes. In 2000, the Clinical Laboratory Standards Institute (CLSI) published document C29A2 indicating that Sodium and Potassium measurements be standardized to the flame photometry methodology, which is concentration-based instead of activity-based. However, the standardization is based on the assumption that the sample is a normal sample. A normal sample is defined by several factors, including the assumption that the mass concentration of plasma water is 0.93 kg per liter, plasma total CO<sub>2</sub> being 24 mmol per liter, plasma pH 7.40, and albumin, total protein, cholesterol, and triglycerides all have its defined means and ranges.

Therefore, after the standardization, the sodium activity as measured by ISE of 154 mmol per kg water is converted to a plasma concentration of 143 mmol per liter, and it is reported in that manner.

**Slide 8:**

The phenomena that sodium and other electrolytes are only present in the water phase and excluded from the solid phase in serum or plasma is called the electrolyte exclusion effect.

This effect has several implications in sodium and other electrolytes measurements. As summarized on this slide, the sodium activity measured by ion-selective electrode is converted to 143 mmol per liter based on the assumption that 7% of sample volume is occupied by non-soluble solids such as lipids and proteins. This negative difference has been long recognized in the chemistry community.

Pseudohyponatremia will occur when samples have more than 7% of solids. This will be discussed in the next slide.

**Slide 9:**

Electrolyte exclusion effect is the main cause for inaccurate reporting of sodium concentrations with indirect ion-selective electrode method when the sample does not have the assumed 93% water phase. In comparison, this represents the typical samples with 93% water phase and 7% solid phase. This slide explains this phenomenon given a hyperlipemic sample that is 80% water phase and 20% solid phase as shown in this sample.

This slide uses dilution factor of 31 to illustrate the effect. Some automated analyzers may use a different dilution factor. The two diagrams on the left side represent a normal sample and a sample with hyperlipemia. During the dilution process for indirect ISE or flame photometry, the volume changed from 1 to 31. In the case of hyperlipemia and hyperproteinemia, as sodium is only present in the water phase, the effective dilution factor for samples will be much higher than what is in a “normal” sample. This effect is equivalent to “short sampling”.

**Slide 10:**

This slide is to put the theory into numbers. The example assumes the sodium activity is 150 mmol/Kg Water. The sodium concentration is measured by Ion-selective electrode in a typical sample with water to solids ratio of 93% to 7% and a hyperlipidemic sample with water to solids ratio of 80% to 20%. In each sample, 15  $\mu$ L of sample is used for a 31-time dilution. The effective dilution factor in the water phase is 33.3 times, and 38.5 times, respectively. As a result, the readings from ISE are 4.50 and 3.89, respectively. However, the calculation in the automated analyzer is based on the theoretical dilution factor, which is based on the assumption that the water contributes to 93% of the total volume or 33.3 effective dilution factor. Therefore, the calculated ISE results are 150 mmol/kg water and 139.6 mmol/Kg water, respectively. After the standardization process based on 93% water phase, the final reports show 139.5 mmol/L and 120.5 mmol/L for these two samples, respectively.

In the case of the sample with hyperlipidemia, the concentration is underestimated resulting in a seemingly hyponatremia condition. When this happens, we call it pseudohyponatremia.

**Slide 11:**

Then, the question is how to measure sodium concentration in those samples correctly or how to avoid pseudohyponatremia?

There are a couple of options. One is to use ultracentrifuge to remove lipids in the samples and only measure the lipids-free serum portion. Attention should be taken during this process to ensure only infranate was taken for analysis and not include any of the lipid layer. However, ultracentrifugation is not good at removing proteins. In the case of hyperproteinemia, alternate methods should be considered, such as filtering the sample to remove proteins or direct ISE and avoid the dilution step. Since the ISE measures the ion activity, it will give a reading of 150 mmol/Kg water for either sample regardless of the solids proportion. The standardization process will provide the same results as 139.5 mmol/L. Formulas to correct sodium concentration based on calculated serum water have been published, though the experimental validity of this method and the availability of direct ISE have made the direct measurement a favorable choice.

**Slide 12:**

How about other electrolytes? Electrolyte exclusion effect has the similar impact on other electrolytes. Sodium is discussed the most because of the tight sodium reference interval, which results in higher clinical impact of false results. 10% analytical error can result in a sodium concentration to be out of its reference interval, while 10% difference would not necessarily push other analytes out of their reference interval. One caveat is that electrolyte exclusion effect can also cause pseudoHYPERnatremia in samples with less solids (< 7%).

**Slide 13:**

This slide summarizes the different methods that are impacted by the electrolyte exclusion effect. In general, flame photometry, atomic absorption spectrometry, coulometry, and indirect ISE will be affected, while direct ISE, gas electrodes, and freezing point depression are not affected.

**Slide 14:**

In conclusion:

- Na<sup>+</sup> is one of the most frequently measured analytes in chemistry laboratories
- its analysis is standardized to the flame photometry method and assumes water phase is relatively constant at 93% of total volume
- dilution prior to analysis can result in falsely low sodium concentrations in samples with hyperlipidemia and or hyperproteinemia
- increased proteins or lipids concentrations will cause pseudohyponatremia due to electrolyte exclusion effect, and other electrolytes are affected in a similar fashion
- direct ISE is not affected by electrolyte exclusion effect and is the preferred method for the analysis of hyperlipemic and hyperproteinemic samples

**Slide 15: References****Slide 16: Disclosures****Slide 17: Thank You from [www.TraineeCouncil.org](http://www.TraineeCouncil.org)**

Thank you for joining me on this Pearl of Laboratory Medicine on “Pseudohyponatremia.” My name is Yan Victoria Zhang.